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Summary Report: Workshop on the Potential Risks of Antibody-Dependent Enhancement in Human HIV Vaccine Trials

JOHN R. MASCOLA,[1] BONNIE J. MATHIESON,[2] PHILIP M. ZACK,[1] MARY CLARE WALKER,[2] SCOTT B. HALSTEAD,[3] and DONALD S. BURKE[1]

INTRODUCTION

In several human and animal viral diseases, suboptimal immunity to an infecting agent can cause an adverse or enhanced clinical expression of disease.[1-3] This phenomenon of immune-mediated disease enhancement can occur on secondary exposure after natural infection, or after virus-specific immunization. In some diseases (e.g., dengue), there is strong evidence that antibody-dependent enhancement (ADE) of infection of monocyte/macrophage (M/M) cells is the pathophysiological mechanism for enhanced disease.[4] In other viral infections, the pathophysiological mechanism is not understood and therefore the term *immune-mediated disease enhancement* is a more accurate description than ADE.[1]

Concerns about ADE in HIV vaccine trials have been raised because serum from HIV-infected individuals can enhance HIV infection of some types of human cells *in vitro*. Several independent laboratories, using different methodologies, have demonstrated that the level of HIV infection detected *in vitro* increases in the presence of certain HIV-specific antibodies (Abs). Depending on the assay system used and the criteria imposed, as many as 95% of sera from HIV-infected persons can be shown to contain enhancing Abs.[3] Although there is, as yet, no convincing evidence that these Abs play a role in altering the pathophysiology of HIV disease, it is possible that immunization of seronegative individuals with certain candidate HIV vaccines might generate enhancing Abs similar to those found *in vitro* in natural infection. Further, it has been proposed that these Abs might be detrimental rather than protective; that is, produce increased susceptibility to, or severity of, HIV infection or more rapid onset of HIV disease.[3]

Initial information from ongoing phase I/II trials of seronegative volunteers with HIV subunit vaccine candidates indicates that most are immunogenic and well tolerated (P. Fast and M. C. Walker, Division of AIDS, NIAID, personal communication). Sera from vaccinees are being evaluated for a wide range of antibody activities, including the presence of neutralizing and enhancing Abs. Because vaccine efficacy and *in vitro* correlates of protection can be established only in large clinical trials, the *in vivo* significance of such measurements is not yet known. To address the issues related to ADE in HIV vaccine trials, a 2-day workshop was convened December 10 and 11, 1992 by the Vaccine Research and Development Branch, Division of AIDS, NIAID, NIH and the Division of Retrovirology, Walter Reed Army Institute of Research and was hosted by the Health Sciences Division of the Rockefeller Foundation (New York, NY). A group of investigators with expertise in ADE, lentiviral animal models, and HIV vaccine-related issues addressed the question: Does antibody-dependent enhancement of HIV infection pose a genuine risk in human HIV vaccine trials? The major objectives of the workshop were (1) to evaluate the potential risk of immune-mediated disease enhancement in human HIV vaccine trials, (2) to propose *in vitro* and animal experiments that would evaluate this potential risk, and (3) to suggest guidelines for determining the risk of ADE associated with specific candidate HIV vaccines.

To better define the potential risks of ADE in human HIV vaccine trials, the first sessions focused on a review and update of several subjects essential to an accurate assessment of this issue. These included (1) immune-mediated enhancement in viral diseases other than HIV, (2) mechanisms and measurements of HIV ADE *in vitro*, (3) *in vitro* ADE by sera from HIV-infected individuals, (4) *in vitro* ADE by sera from HIV-vaccinated animals and humans, and (5) HIV genetic variation, that is, potential impact of genetic subtypes or serotypes of HIV-1 on the risk of ADE. Because ADE is currently the only well-documented mechanism for immune-mediated disease enhancement, and because ADE can often be measured *in vitro*, this workshop focused on experiments that could correlate *in vivo* disease enhancement with *in vitro* detection of ADE in vaccine studies. In discussion sessions the second day, designs

[1]Division of Retrovirology, Walter Reed Army Institute of Research, Rockville, Maryland 20850.
[2]Vaccine Research and Development Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.
[3]Health Sciences Division, the Rockefeller Foundation, New York, New York.

for future *in vitro* and animal model studies were drafted that would address the gaps in our knowledge about the risks posed by ADE in HIV vaccine trials and provide a basis for decision making in HIV vaccine development.

Immune-mediated enhancement of viral diseases other than HIV

Immune-mediated disease enhancement in several viral diseases, including lentiviral diseases other than HIV, was reviewed in a session chaired by S. Halstead (Rockefeller Foundation). There are examples of enhanced clinical disease in both vaccinated and previously exposed animals and humans. These are briefly reviewed below. A more comprehensive discussion of this subject occurs elsewhere.[1-3]

Human viral diseases. The human disease for which immune-mediated enhancement is best understood is dengue. Dengue viruses, members of the Flaviviridae family, occur as four antigenically distinct serotypes. After a short period of cross-protection, individuals infected with one serotype are fully susceptible to infection with other serotypes. Dengue fever, usually a benign self-limited febrile illness, can occasionally occur as a severe syndrome called dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Such severe manifestations almost always occur in individuals with preexisting Abs to one dengue serotype (either from prior infection or of maternal origin), and subsequent exposure to a different serotype.[4] *In vitro*, heterotypic dengue Abs can enhance dengue virus infection of human M/M cells.[5] In a prospective study of school children in Thailand, *in vitro* enhancing activity of serum was a powerful predictor of DHF/DSS among dengue-immune children.[6] This was the first human study to establish a clear link between serum ADE and enhanced clinical disease. It is now widely accepted that ADE plays an important role in dengue infection, and the theoretical risk that dengue vaccination might potentiate more severe disease has delayed the development and testing of dengue vaccines.[7] Of note, there are several examples among the flaviviruses in which serum ADE activity has been demonstrated *in vitro* (against both homologous and heterologous flavivirus species) despite the fact that *in vivo* disease enhancement does not occur.[5,8] In fact, successful vaccines for yellow fever and Japanese encephalitis have been used in millions of persons and do not appear to enhance infection of either homologous or heterologous flaviviruses.[3]

Immune-mediated disease enhancement has also been described in individuals vaccinated against respiratory syncytial virus (RSV). Following immunization with a formalin-inactivated whole-virion RSV vaccine, children were at increased risk of severe RSV disease compared with control groups. This effect was dramatically illustrated in four parallel studies conducted between 1966 and 1967.[9] Subsequent evaluation of vaccinee serum from one of the studies revealed high titers of "non-nonfunctional" (i.e., nonneutralizing) Abs compared to naturally infected children.[10] Although there are no reports of enhancement studies on sera from the vaccinated children, particularly those with enhanced clinical disease, human sera containing RSV-specific Abs can enhance RSV infection of the macrophage cell line U937.[11] Furthermore, RSV can replicate *in vitro* in human peripheral blood M/M cells.[12]

Another example is the killed measles vaccine, which was administered to over 500,000 children between 1963 and 1967. It was withdrawn when it became evident that it predisposed some recipients to an exaggerated illness referred to as atypical measles syndrome.[13] The pathogenesis of this syndrome is not known and *in vitro* ADE was not evaluated. Therefore, in RSV and measles, although immune-mediated enhancement of disease clearly occurs, the mechanism of enhanced disease and the possible role of ADE of infection are not understood.

Animal viral diseases. Feline infectious peritonitis virus disease (FIPV) is a coronavirus that causes a fatal multisystem pyogranulomatous disease in kittens and cats. F. Scott (Cornell University, Ithaca, NY), reviewed the evidence for immune-mediated disease and the mechanism of enhancement of FIPV. The primary target for viral replication *in vivo* is believed to be the macrophage.[14] Cats with active or passive immunity to FIPV often develop an accelerated and more fulminant disease course on FIPV challenge than seronegative cats, and kittens immunized with a recombinant vaccinia virus expressing the spike protein of FIPV succumbed earlier than did control animals.[15] The role of Abs in promoting more severe disease has been documented by passive transfer of FIPV-reactive immune sera or purified immunoglobulin (Ig) to seronegative cats.[16] Furthermore, *in vitro* FIPV infection of primary feline peritoneal macrophages is enhanced in the presence of FIPV Abs.[14] Although definitive experiments demonstrating that *in vitro* ADE is predictive of exaggerated clinical disease have not yet been performed, FIPV vaccine development has been hindered by the *in vitro* and *in vivo* observations of immune enhancement of FIPV infection.

There have also been reports of disease enhancement after vaccination of animals with killed whole-virus vaccines for bluetongue disease of cattle and Aleutian disease of mink.[3] Of particular interest is a study of Aleutian disease virus in which 8 of 10 vaccinated mink, but none of 4 control animals, developed Aleutian disease after oral challenge with a standardized infectious dose of virus.[17] This is one of the few studies clearly demonstrating that immune-mediated enhancement can lead to an increased susceptibility to infection as well as a more severe disease course.

Lentiviral diseases. Equine infectious anemia virus (EIAV) is a lentiviral infection of horses that generally causes a syndrome of fever, anorexia, and anemia with cyclical recurrences during the first year of disease. Subsequently, horses may become asymptomatic or develop a chronic wasting syndrome. Data were presented that demonstrated *in vivo* immune-mediated disease enhancement of vaccinated ponies challenged with EIAV (R. Montelaro, University of Pittsburgh, PA). In experiments with an inactivated whole-virus vaccine, horses challenged with homologous virus were protected from infection whereas animals challenged with a heterologous viral strain became infected but were protected from disease.[18] In contrast, immunization with a baculovirus-expressed EIAV envelope glycoprotein vaccine generated no protection against homologous viral challenge and significant enhancement of disease severity was observed when the immunized ponies were challenged with a heterologous EIAV strain. No *in vitro* data on ADE in sera from vaccinated ponies are yet available.

Caprine arthritis-encephalitis virus (CAEV) and visna virus are ungulate lentiviruses. CAEV causes a gradually progressive debilitating arthritis in goats, whereas visna virus causes a chronic progressive paralyzing disease of the central nervous

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JOHN R. MASCOLA, B. MATHIESON, P. ZACK, M. WALKER, S. HALSTEAD, D. BURKE

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13. ABSTRACT (Maximum 200 words)

In several human and animal viral diseases, suboptimal immunity to an infecting agent can cause as adverse or enhanced clinical expression of disease. This phenomenon of immune-mediated disease enhancement can occur on secondary exposure after natural infection, or after virus-specific immunization. In some diseases(e.g. dengue), there is strong evidencethat antibody-dependent enhancement(ADE) of infection of monocyte/macrophage(M/M) cells is the pathophysiological mechanism for enhanced disease. In other viral infections, the pathophysiological mechanism is not understood and therefore the term "immune-mediated disease enhancement" is a more accurate description than ADE.

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system in sheep. P. Jolly (University of Alabama, Birmingham), reviewed *in vitro* and *in vivo* data for ADE in these diseases. Goats vaccinated with a formalin-inactivated CAEV vaccine developed more rapid and severe arthritis after CAEV challenge than did control goats.[19] Furthermore, Ab to CAEV and visna virus can enhance viral binding and penetration into sheep macrophages, although, surprisingly, there is no increase in production of virions.[20] There are no studies specifically correlating *in vitro* ADE with *in vivo* immune-mediated enhancement.

Unlike CAEV and EIAV, *in vivo* immune enhancement has not been reported in simian immunodeficiency viruses (SIV) disease. Numerous vaccination and challenge experiments have been done with SIV (reviewed by A. Schultz, Division of AIDS, NIAID and M. Murphy-Corb, Delta Regional Primate Center, Tulane University, Covington, LA). Immunization of monkeys with inactivated whole virus, glycoprotein subunit, and peptide products has not yielded evidence for enhanced clinical disease on viral challenge with either homologous or heterologous viral strains. However, it is important to note that experiments have not been specifically designed to look for disease enhancement. Sera from some vaccinated monkeys do contain enhancing Abs (discussed in more detail below in In Vitro Data for Antibody-Dependent Enhancement in Sera from Vaccinated Animals and Humans). Also, sera from SIV-infected rhesus macaques can enhance SIV infection of certain transformed cell lines.[21]

In summary, with the probable exceptions of dengue and FIPV, the immunopathological mechanism for immune enhancement in several viral diseases is not well established. However, a common theme emerges in dengue, measles, FIPV, and some lentiviruses, that cells of M/M lineage are an important host cell for viral replication *in vivo*. In addition, *in vitro* enhancement of viral infection of M/M cells has been demonstrated in dengue, RSV, FIPV, and the lentiviruses. Thus, infection of M/M cells may be an important determinant that places a host at risk for immune-mediated enhanced disease. In relating this evidence for immune-mediated enhancement to HIV, it may be important to note that cells of M/M lineage also play an important role in the pathology of HIV infection. Therefore, experiments with strains of HIV or SIV displaying M/M tropism[22–24] may be particularly informative for evaluation of immune enhancement. Moreover, strains of HIV that are not macrophage tropic may become capable of infecting macrophages through ADE.

Measurement and mechanisms of HIV antibody-dependent enhancement in vitro

In vitro, ADE generally refers to the ability of Abs to increase viral growth in cultured cells by facilitating viral infection of these cells. The enhancing (or neutralizing) activity measured with a given serum depends on multiple factors specific to the assay system employed. Variables such as the target cells, the distribution of receptors on the target cells, presence or absence of complement, serum dilutions tested, and strain of HIV-1 used can all affect the final outcome.

CD4 is known to be an important receptor for HIV infection of human leukocytes; however, direct binding to CD4 may not be the only route of entry for HIV.[25] Most reports of *in vitro* ADE of HIV infection have demonstrated the requirement that target cells express either the immunoglobulin Fc receptor (FcR) or complement receptors (CRs) in addition to CD4. In addition to direct binding of HIV gp120 to the CD4 receptor, the virus has been shown to bind and enter cells after attachment to FcR and CR. The expression of FcR and CR on human cells of the M/M lineage is well established. The distribution of these receptors on other cell types is highly variable (reviewed by M. Fanger, Dartmouth Medical School [Hanover, NH], and G. Spear, Rush Presbyterian Medical Center) and is summarized in Table 1. These cellular receptors are also found on subtypes of cells such as follicular dendritic cells of the lymph node and blood-borne dendritic cells. These two types of specialized cells may be of particular importance in HIV infection.[26–29]

In vitro assays for ADE of HIV infection usually employ, as target cells, neoplastic cell lines expressing some combination of FcR, CR, and CD4. Like assays for Ab-mediated neutralization, results are often expressed as the highest serum titer giving a predetermined effect on viral growth. In most reports on ADE, there is a biphasic response based on serum dilution, that is, at lower (undiluted) concentrations viral neutralization is ob-

TABLE 1. EXPRESSION OF IMMUNOGLOBULIN AND COMPLEMENT RECEPTORS ON VARIOUS CELL TYPES *in Vivo*[a]

	Main cellular expression							
Receptor class	*T cell*	*B cell*	*NK*	*PMN*	*Macrophage*	*Monocyte*	*RBC*	*FDC*
$Fc_\gamma RI$					*	*		
$Fc_\gamma RII$		*		*	*	*		
$Fc_\gamma RIII$			*	*	*			
$Fc_\mu R$	*							
$Fc_\epsilon RII$		*				*		*
$Fc_\alpha R$				*	*	*		
CR1		*		*	*	*	*	*
CR2		*						*
CR3			*	*	*	*		*
CR4				*	*	*		

[a] Adapted from Ref. 3. NK, Natural killer cell; PMN, polymorphonuclear leukocyte; RBC, red blood cell; FDC, follicular dendritic cell.

served, whereas enhancement is seen at higher dilutions. Enhancement has also been reported as an "enhancement ratio" (ratio of viral yield in the presence of Ab to that in the absence of Ab) for each serum dilution. In different assay systems, the magnitude of enhancement of HIV infection (measured by level of viral expression) has ranged from barely 2-fold to greater than 100-fold.[30] Likewise, the highest serum dilution showing ADE ranges from 10^1 to 10^5. The method of measuring and expressing ADE activity can be of critical importance in attempting to correlate *in vitro* ADE with an *in vivo* effect. For example, in secondary dengue infections, serum-enhancing activity of undiluted sera was a significant risk factor for severe dengue illness when human peripheral blood mononuclear cells (PBMCs) were used as indicator cells. In contrast, end-point ADE titer (highest titer showing enhancing activity) was not predictive, and paradoxically was higher in children without severe illness.[6]

The relationship between neutralizing and enhancing Abs is of particular interest. At any given serum dilution, the overall effect on viral growth *in vitro* is likely to be the net sum effect of neutralizing and enhancing Abs. In complex mixtures of cells, such as exist *in vivo* in humans, or even *in vitro* in PBMCs, Abs in a given serum may protect some cells from infection while simultaneously increasing the rate of infection of others. Even without a net increase in the number of cells infected, enhancing Abs may alter pathogenesis by retargeting virus to different populations of cells. These issues can be partly addressed by using selected cells as targets for assays or by selecting a virus with specific cell tropism (e.g., M/M cells) features. Attention to the nuances of such *in vitro* measurements will ultimately facilitate our ability to correlate *in vitro* measurements with clinical outcomes of trials.

In vitro *data for antibody-dependent enhancement by sera from HIV-infected individuals*

Several groups of investigators have independently demonstrated ADE activity in sera from HIV-infected individuals. In 1987, Robinson *et al.* noted that a factor intrinsic to HIV-seropositive sera caused an increase in cytopathic effect and syncytium formation in human transformed cells (MT-2).[31] Subsequent evaluation (reviewed by E. Robinson, University of California, Irvine, CA) demonstrated that Ab and complement were necessary to mediate the enhancing effect of serum and that the increased viral expression seen in this assay was a result of increased viral binding to MT-2 cells.[32,33] This complement-mediated ADE (C′-ADE) requires cell receptors for both CD4 and CR type 2 (CR2)[34] and was seen with the IIIB and RF strains of HIV-1. Sera from 95% of HIV-infected individuals (all stages of disease) show ADE when assayed in this manner.[30] Most sera demonstrate neutralizing activity at high concentrations and C′-ADE at subneutralizing concentrations. Interestingly, complement depletion of sera eliminated ADE activity and usually revealed significant serum neutralization activity measured in the same assay. M. Wainberg (McGill University, Montreal, Quebec, Canada) reviewed data that C′-ADE also occurs when Epstein–Barr virus (EBV)-transformed human B cells are used at target cells.[35,36]

A second type of ADE requiring the FcR rather than the CR has also been described in which HIV-specific Ab, but not complement, is required for enhancement to occur. FcR-mediated ADE (FcR-ADE) of infection of the human monocytic cell line U937 has been demonstrated using the IIIB strain of HIV-1.[37–41] Others have reported FcR-ADE of infection of primary human lymphocytes and macrophages, using several HIV-1 isolates grown in PBMCs.[42,43] In these assays, low levels of FcR-ADE have been found in most HIV immune sera,[37] and like C′-ADE, serum-enhancing activity usually occurs at subneutralizing concentrations. In contrast, Shadduck *et al.* found that HIV-1 infection of human monocytes and peritoneal macrophages was not facilitated by any of 14 human HIV-1-seropositive sera.[44] This result was consistent for four viral isolates and in the presence or absence of complement. Most reports of FcR-ADE have described a requirement for the CD4 receptor; however, in one report, using primary human macrophages, CD4 was not found to be necessary for FcR-ADE.[42] Some of the major parameters that vary among *in vitro* enhancement assays are listed in Table 2.

Efforts have attempted to define whether there are specific epitopes responsible for ADE. Robinson *et al.* have demonstrated that human monoclonal antibodies (MAbs) to the immunodominant region of gp41 can mediate C′-ADE.[45,46] Evaluation of gp120 MAbs for similar C′-ADE activity is in progress. A human monoclonal antibody that binds to a conformational epitope in the carboxyl half of gp120 has been reported to

TABLE 2. COMPARISON OF *in Vitro* ENHANCEMENT ASSAY METHODOLOGIES

Viral strain	*Target cells*[a]	*C′-ADE vs. FcR-ADE*	*CD4 receptor required*	*Magnitude of maximum effect*[b]	*Serum dilutions*[c]	*Ref.*
IIIB,RF	MT-2	C′-ADE	Yes	100-fold	10^1–10^5	30, 31, 34
IIIB + clinical isolate	EBV/B cells	C′-ADE	Yes	10-fold	10^2–10^3	35, 36
RF	MT-2	C′-ADE	Yes	10-fold	10^2–10^3	33
IIIB	U937	FcR-ADE	Yes	4-fold	10^3–10^6	37, 38
IIIB	U937	FcR-ADE	Yes	4-fold	10^3–10^5	39
IIIB	U937	FcR-ADE	Yes	3-fold	10^3–10^4	40
IIIB	U937	FcR-ADE	Yes	—	$>10^4$	41
Clinical isolates	PBMCs, M/M	FcR-ADE	No	10-fold	10^1–10^4	42, 43

[a] EBV/B cells, EBV-transformed human B cells; M/M, primary human monocyte/macrophages.
[b] Maximum increase in viral growth in the presence of enhancing serum.
[c] Serum dilutions for which ADE is described.

mediate FcR-ADE.[47] Further evaluations of *in vitro* correlates of immune protection and enhancement for HIV are needed to assess the biological significance of specific epitopes in a vaccine candidate.

Because our central concern is whether ADE plays a role in vaccinated individuals exposed to primary "field" isolates of HIV, the analysis of such isolates is of primary importance. C. Hanson (California Department of Health, Berkeley, CA) presented data that HIV grown in human PBMCs is more susceptible to C'-ADE and less susceptible to neutralization than is HIV grown in a transformed cell line (H9). Furthermore, antiserum–virus pairs showing strong C'-ADE when assayed on MT-2 cells do not show C'-ADE in a PBMC indicator system. S. Kliks (University of California, San Francisco, CA) reported experiments using primary PBMCs for both viral growth and indicator cells; replication of a M/M-tropic HIV isolate could be enhanced by HIV immune sera whereas growth of the T cell-tropic isolate (HIV-SF2) could not. Clearly, the type of cells used as indicators for viral replication, as well as the type of cells used to grow HIV-1 in culture, can affect *in vitro* neutralization and enhancement results. The explanations for these observations will require further study.

Several limited studies evaluating the relationship between serum ADE activity and stage of disease have found no consistent link for *in vitro*-detected ADE with HIV disease progression. Montefiori *et al.* found no correlation between titers of complement-dependent enhancing antibodies and stage of disease in 54 HIV-positive individuals in the multicenter AIDS cohort study group.[48] In contrast, Toth and colleagues found serum C'-ADE activity in 12 of 19 symptomatic vs. 4 of 20 asymptomatic HIV-infected patients.[49] Both of these studies were done using the IIIB strain of HIV-1 and transformed cells as targets. Homsy *et al.*, using PBMCs as target cells, reported that serum-enhancing activity (FcR-ADE) against homotypic virus was present in 1 of 5 asymptomatic vs. 6 of 11 symptomatic HIV-infected patients. Furthermore the serum-enhancing activity of three of five individuals followed for 1–3 years appeared to increase over time.[43] As noted, the assay methodology differed significantly among these studies; in the C'-ADE studies, the IIIB strain of HIV was used to infect transformed cell lines, whereas in the FcR-ADE study homotypic virus was used to infect human PBMCs. Clearly further studies are required to elucidate the possible role of ADE in the pathogenesis of HIV *in vivo*.

In vitro *data for antibody-dependent enhancement by sera from HIV-vaccinated animals and humans*

Although it is clear that naturally infected humans have enhancing antibodies to HIV, the type of immune response generated after vaccination is more pertinent to the question of ADE risk in vaccinees. In 1989, Homsy *et al.* reported that serum from a guinea pig hyperimmunized with HIV-SF128A could enhance HIV infection of PBMCs.[42] In addition, data showing that sera from rabbits immunized with several types of HIV gp120 or V3 loop peptides enhanced HIV-IIIB infection of U937 cells[50,51] were reviewed by S. Jiang and R. Neurath (New York Blood Center, New York, NY).

In human vaccinees, Bernard *et al.* evaluated three individuals immunized with vaccinia virus expressing gp160 and boosted with autologous cells infected *in vitro*. Heat-inactivated sera at a dilution of 1:100 from all three demonstrated neutralizing and not enhancing activity of HIV-IIIB infection of primary human macrophages.[52] F. Ennis (University of Massachusetts, Amherst) and D. Montefiori (Vanderbilt University, Nashville, TN) reviewed results from two NIAID-sponsored phase I vaccine trials. Of 24 seronegative volunteers vaccinated with a baculovirus-expressed recombinant gp160 product, after the third boost 6 had low-titer C'-ADE activity measured in MT-2 cells,[53] none of 13 sera obtained after the second boost demonstrated FcR-ADE measured in U937 cells.[54] In another trial evaluating primary vaccination with a recombinant vaccinia virus expressing gp160 followed by booster immunization with gp160, C'-ADE activity was detected in 7 of 10 vaccinees. Several other candidate HIV-1 vaccines are currently undergoing phase I and/or phase II testing through the AIDS Vaccine Evaluation Group (AVEG) of DAIDS, NIAID. Studies are ongoing or planned to evaluate the sera from vaccinees for the presence of neutralizing and enhancing Abs.

Of note, C'-ADE has been demonstrated in rhesus macaques vaccinated with whole inactivated SIV vaccines, or with glycoprotein or core protein-enriched subunit vaccines.[55] In these SIV challenge studies, as in dengue in children,[6] high serum titers of enhancing Abs correlated better with protection than with infection.[55]

Although studies of sera from individuals vaccinated with several HIV-1 candidate AIDS vaccines are in preliminary stages, it is likely that, similar to findings in HIV-infected individuals, some *in vitro* assays will detect ADE in some sera from vaccinees. Because ongoing analysis of sera will be from phase I/II immunogenicity trials of HIV vaccinees, we do not expect to determine *in vivo* correlates for these *in vitro* data. Therefore, interpretation of the significance of these results will be limited until further in vivo studies are performed. This issue is discussed further in Evaluation of the Antibody-Dependent Enhancement Risk Associated with Particular HIV Vaccine Candidates.

HIV genetic variation: potential impact of genetic subtypes or serotypes of HIV-1 on the risk of antibody-dependent enhancement

DNA sequence analysis of HIV-1 has demonstrated marked genetic diversity among isolates collected worldwide; phylogenetic tree analysis reveals at least five genetically distinct clades or subtypes of HIV-1.[56,57] The immunological significance and the correlation of antigenic subtypes to these genetic subtypes are not well understood. (J. Mascola, Walter Reed Army Institute of Research [WRAIR]) presented data that viruses representing two distinct genetic subtypes can be distinguished using a neutralizing antibody assay.[58] Thus, it is possible that antigenically distinct serotypes of HIV-1 exist. Current vaccine candidates are based on prototype viral strains (HIV-MN, HIV-IIIB/LAI, HIV-SF2), which are all representatives from a single genetic subtype. Therefore, international vaccine trials planned with current vaccine candidates could result in immunization with a serotype of HIV that is different from that prevalent in the population of vaccinees. The dengue and EIAV experience of enhanced disease after infection with a heterologous serotype heightens the concern that an analogous scenario could occur in HIV vaccine trials.

Summarizing the risk of antibody-dependent enhancement in HIV vaccine trials

In summary, there are several factors which, when viewed together, raise concern that ADE of HIV infection is a potential risk in human HIV vaccine trials. We know that immune-mediated disease enhancement occurs in several viral diseases, including lentiviral diseases (e.g., EIAV). Manifestations of enhancement include increased viremia, shortened incubation period, altered pathogenesis, more severe disease outcome, and an increased susceptibility to infection. In dengue and FIPV, evidence indicates that ADE of M/M cells *in vivo* is the probable mechanism of enhanced disease. Viral replication in host M/M cells is characteristic of most of the diseases for which immune enhancement has been described, and is well described in lentiviral diseases, including HIV. Sera from HIV-1-infected persons contain antibodies that enhance the growth of HIV *in vitro* and it is reasonable to expect that some sera from HIV-1 vaccinees will also contain enhancing antibodies. Finally, the known genetic diversity of HIV-1 worldwide, the possibility of antigenically distinct serotypes, and the fact that current vaccine candidates represent a single genetic subtype, heighten concern that immunization could enhance heterologous subtypes of HIV-1 (similar to what occurs in dengue and EIAV).

These concerns regarding ADE in vaccine trials are partially balanced by the observation that enhanced disease has not yet been observed in challenge experiments of SIV-vaccinated monkeys, despite the fact that in some cases complement-dependent enhancing Abs were present in high titer at the time of challenge.[55] Also of note, ADE of virus growth *in vitro* has been described in numerous viral diseases and often has no *in vivo* correlate.[3] Because we currently lack clinical evidence for ADE in HIV disease, there are no *in vivo* correlates for the *in vitro* observations of ADE. Therefore, it is not possible to accurately assess the significance of *in vitro* measurements in naturally infected or immunized individuals. An HIV animal model is required to advance our understanding of the potential risk of ADE in HIV vaccine trials. Such HIV/SIV animal model experiments are discussed in the next section. Our knowledge of immune-mediated enhancement in other viral diseases, especially lentiviral diseases, and data for *in vitro* ADE in HIV disease, do indicate that well-directed studies are needed to further address the risk of ADE in HIV vaccine trials. However, there is currently insufficient evidence to halt or delay the development of specific HIV vaccine candidates or early clinical trials with these candidates. The next two sections suggest, in general terms, key *in vitro* and animal experiments that could better define the potential risk of ADE, including a discussion of the evaluation of individual vaccine candidates prior to large-scale efficacy trials.

STUDIES THAT WOULD ADDRESS THE POTENTIAL RISK OF ANTIBODY-DEPENDENT ENHANCEMENT IN HIV VACCINE TRIALS

The second day of the workshop was devoted to an open discussion of proposals that would address the risk of ADE (or immune-mediated enhancement) in HIV vaccine trials. Discussion chairpersons were P. Fast and A. Schultz (Division of AIDS, NIAID), and D. Burke (WRAIR).

Epidemiological surveys in HIV-infected individuals

S. Halstead discussed the possibility that if ADE of HIV infection occurs, it might be found in instances in which an individual is exposed to HIV in the presence of anti-HIV antibodies. HIV infection acquired by blood (plasma) transfusion and HIV acquired neonatally by infants who circulate maternal HIV antibodies are examples that comply with the conditions under which anti-HIV antibody should be present and ADE might occur. Careful epidemiological studies of individuals exposed to HIV in this manner, with matched controls, might answer the question of whether ADE occurs in HIV disease and what its manifestations are. Negative studies would be reassuring with respect to the putative risk of ADE after HIV vaccination.

Examples of such epidemiological studies were discussed, including (1) retrospective evaluations of transfusion-infected individuals comparing plasma-containing vs. plasma-free products, and (2) mother/infant studies attempting to correlate the risk of HIV transmission with *in vitro* measurement of neutralizing and enhancing antibodies.

HIV/SIV animal model experiments

If ADE of HIV infection occurs *in vivo*, vaccinees with partial or incomplete immunity might be more susceptible to HIV infection, or manifest a more severe disease course. In the setting of a large HIV vaccine efficacy trial, probably the most serious adverse outcome is an increased incidence of HIV infection in vaccinees. Therefore, animal experiments that would address this risk are of primary importance. With regard to assessment of the risk of ADE in HIV vaccine trials, HIV/SIV animal models have two primary objectives: (1) to determine if susceptibility to infection is enhanced by active or passive immunization prior to viral challenge, and (2) to determine if the progression of disease is augmented by active or passive immunization prior to viral challenge.

Experiments addressing the first issue would determine if the animal ID_{50} (50% infectious dose) of the challenge virus is lowered after active or passive immunization. Susceptibility to heterologous as well as homologous strains of challenge virus should be considered and close measurements made of the level of circulating neutralizing and enhancing antibodies present at the time of challenge. Passive immunization studies should be designed to establish circulating antibody levels in animals at concentrations shown to enhance viral growth *in vitro*. Studies designed to evaluate potential enhancement of disease progression should be able to make multiple measurements of the humoral immune response and assess several outcome measures, which include (1) shortened incubation period from time of infection to onset of viremia, onset of clinical disease, and mean time to death, (2) enhanced viremia or viral burden in circulating cells or tissues, and (3) altered pathogenesis, that is, alteration in the number or type of infected cells (e.g., cells of M/M lineage).

In both scenarios for ADE, challenge experiments with heterologous as well as homologous virus should be performed. Specific heterologous challenge experiments should be designed to model the possibility of vaccinees encountering a strain of HIV that is genetically divergent (i.e., a different genetic subtype) from that on which the vaccine was based. If enhance-

ment of HIV infection is demonstrated *in vivo*, *in vitro* correlates should be sought that would be predictive of *in vivo* ADE.

Current animal models available

The ideal animal model for evaluating the risk of ADE in HIV vaccine trials would have several characteristics: (1) ability to support productive infection with HIV-1 field isolates representing divergent genetic subtypes, (2) development of an immunodeficiency syndrome similar to AIDS in humans, and (3) availability of sufficient numbers of animals. Several animal models using HIV-1 or SIV were discussed with respect to their utility in evaluating the risk of ADE in HIV vaccine trials. These are briefly summarized below.

HIV-1 animal models. Currently the only animals that are susceptible to infection with HIV-1 and could be utilized to study HIV-1 immune-mediated enhancement are the chimpanzee and the severe combined immunodeficiency (SCID) mouse. Although there has been a report of HIV-1 infection of pig-tailed macaques (*Macaca nemestrina*), this model will require further development before it can be used in enhancement studies.[59]

The chimpanzee is the only primate species available that may be infected with HIV-1 and therefore employed for studying protective vaccine efficacy. Unfortunately, there are several major limitations to this model even though infected chimpanzees seroconvert with antibodies to all major HIV-1-encoded structural proteins and HIV-1 can be isolated from their PBMCs. Chimpanzees are not susceptible to productive infection with all HIV-1 isolates *in vivo*. The only two strains of HIV-1 successfully titrated in chimpanzees *in vivo* are HIV-IIIB/LAI and more recently HIV-SF2 (A. Schultz, Division of AIDS, NIAID, NIH, Bethesda, MD), and long-term persistent viremia in chimpanzees has been documented only with variants of HIV-IIIB/LAI. In fact, virtually all other HIV-1 isolates tested replicate poorly, if at all, in chimpanzee PBMCs (P. Zack, WRAIR). This restriction seriously limits the capability to perform immune enhancement challenge experiments using specific HIV-1 field isolates, and precludes experimental studies using heterologous viral challenge groups. Another disadvantage is that HIV-1 infection produces no clinical disease in chimpanzees. This limitation restricts utilization of the model to determine if there is immunologically mediated enhanced disease progression. Finally, chimpanzee experiments must be limited to small numbers of animals and are expensive to conduct. These drawbacks may limit the utility of the HIV chimpanzee model for study of *in vivo* enhancement.

A second animal model for HIV-1 infection has been described using SCID mice reconstituted with human PBMCs (hu-PBL-SCID) or human fetal thymus, lymph node, or liver tissue (SCID-hu). In both types of SCID mice models, transplanted human tissue and IgG have been detected for up to 6–12 months, and in both models SCID mice are susceptible to infection with HIV-1.[60,61] Potential advantages of this model include the ability to infect mice with most HIV-1 isolates, to reconstitute mice with cells from human vaccinees, and to perform passive antibody transfer experiments. Unfortunately there are several limitations to the HIV-SCID mouse model. The extent of reconstitution may vary with the human donor source, implying that the number of human cells available for infection will also differ with the donor. Additionally, high concentrations of virus must be used to infect each mouse and there are few descriptions of *in vivo* titrations of HIV-1 in transplanted SCID mice. Finally, as in the chimpanzee model, there is no clinical disease in HIV-1-infected SCID mice, making evaluation of disease progression impossible.

SIV primate model. SIV is the primate lentivirus most closely related to HIV-1, and is genetically closely related to HIV-2. It shares many biological properties with HIV-1, including CD4 lymphocyte and M/M tropism, and cell entry via the CD4 receptor.[62] Experimental inoculation of SIV into several macaque species induces an immunodeficiency syndrome strikingly similar to AIDS in human beings.[63] There are several different well-characterized strains of SIV, including a variety of pathogenic molecular clones, each of which produces a slightly different clinical syndrome. Many of these viral strains have been titrated *in vivo* in macaques and are readily available for experimental utilization. In the SIV model, therefore, challenge viruses may be chosen that vary with respect to pathogenicity, viral burden, and mean time to death. Furthermore, by using molecular clones, specific epitopes responsible for enhancement can be mapped and the corresponding sequences in the clone modified as desired.

The major limitations of the SIV model are the inability to evaluate specific HIV-1 vaccine candidates (comparable SIV analog vaccines must be constructed) and its inability to provide specific information concerning immunological determinants of immune-mediated enhancement of HIV-1 infection. As previously discussed, disease enhancement has not been observed in numerous experiments designed to evaluate vaccine efficacy in SIV. However, additional experiments designed specifically to detect manifestations of *in vivo* enhancement such as increased susceptibility to a low-dose infection or advanced progression of disease would be informative. Certainly, the absence of *in vivo* enhancement in SIV studies would be reassuring with respect to the potential risk of ADE in HIV vaccine trials.

Chimeric SIV/HIV (SHIV) primate model. Two groups (Li *et al.*, and Shibata *et al.*) have reported the productive infection of cynomolgus macaque monkeys with chimeric viruses that express the Gag and Pol proteins from SIV, and the Env proteins of HIV-1.[64,65] These chimeras remain infectious for nonhuman primates because the *in vivo* host range is predominantly determined by Gag and Pol, and because HIV and SIV both use the same receptor to infect host cells. Because envelope glycoproteins are believed to be the principal targets of protective immunity in lentiviral diseases,[66,67] they are likely to be equally important for immune-mediated enhancement. Theoretically, therefore, the SHIV model could overcome the limitations of the SIV model by allowing infection of macaques with SHIV molecular constructs containing *env* genes from genetically divergent HIV-1 viruses. This model, however, still remains under development. Although macaques were successfully infected and antibodies detected to both HIV-1 envelope and SIV Gag proteins, virus was difficult to recover beyond 4 months postinoculation. Additionally the absence of clinical signs of immunodeficiency in any of the infected macaques demonstrated that the SHIV construct was less virulent than the parent SIV clone. Construction of SHIV chimeric viruses is difficult because the SIV *env* gene normally overlaps the SIV regulatory genes and introduction of HIV-1 *env* must be accompanied by certain HIV-1 regulatory genes. Furthermore it is not

clear whether SHIVs constructed using *env* from other/all HIV-1 strains will retain the capability to infect macaques *in vivo*.

Currently, therefore, no animal model is available that is permissive for productive infection with multiple HIV-1 isolates and that subsequently develops an immunodeficiency syndrome. Despite its limitations, the SIV model fulfills many of the criteria required to study immune-mediated enhancement and is immediately available. The concern that immune-mediated enhancement is a risk factor for HIV vaccine trials would be significantly reduced if prospectively designed experiments using SIV fail to find evidence demonstrating this phenomenon. Theoretically, the chimpanzee model could be used to evaluate enhanced susceptibility to infection after vaccination, although available viral strains for challenge are limited. With further development, other animal models using HIV-1 or its genes may prove to be useful.

What further in vitro *antibody-dependent enhancement experiments should be pursued?*

To establish *in vitro* correlates of vaccine protection or enhancement, *in vitro* experiments should be done as part of well-designed animal and human trials. Evaluation for both neutralizing and enhancing antibodies is a critical part of studies designed to evaluate *in vivo* ADE. Assays done in primary human PBMCs or M/M cells are most likely to be informative, although without *in vivo* correlates, no assay should be dismissed as irrelevant. Evaluation of sera from phase I HIV-1 vaccine volunteers is discussed in the next section.

EVALUATION OF THE ANTIBODY-DEPENDENT ENHANCEMENT RISK ASSOCIATED WITH PARTICULAR HIV VACCINE CANDIDATES

As stated above, particular vaccine candidates ideally would be evaluated in an HIV animal model that would permit challenge with divergent HIV-1 field isolates. As we await progress in development of HIV-1/SHIV animal models, we should carefully obtain *in vitro* data on enhancing and neutralizing antibody activity in sera from HIV-1 vaccine volunteers. Again, assays performed in primary human PBMCs and M/M cells are essential. Specifically, most field isolates of HIV-1 can be propagated only in primary human cells. Therefore, studies evaluating the activity of neutralizing and enhancing antibody against various field isolates can be performed only in these cells. Given our knowledge of diverse genetic subtypes of HIV-1, vaccinee sera should be evaluated for the presence of neutralizing/enhancing antibodies against homologous and heterologous strains of HIV-1, including strains that are genetically divergent from the vaccine strain. Of particular importance are data regarding functional antibody against the viral strains prevalent in the population in which a vaccine will be studied.

Given that *in vitro* correlates predictive of protection and enhancement *in vivo* are not known, specific *in vitro* ADE data from vaccinees should generally not deter development of a particular vaccine candidate. However, specific comparisons of vaccinee sera for neutralizing and enhancing activity against various viral strains may be important. If, for example, sera display neutralizing activity against genetically similar viruses and no neutralizing activity, but rather enhancing activity, against genetically divergent HIV-1 isolates, we would be hesitant to test that product in a population likely to be exposed to the divergent strain of HIV-1. In such a scenario, careful animal model experiments, as discussed above, would be required to further evaluate the potential risks and benefits of the vaccine product.

SUMMARY

Concern that ADE of HIV infection could occur *in vivo*, as a result of HIV immunization, has arisen for several reasons. Immune-mediated disease enhancement occurs in several human and animal viral diseases, including lentiviral diseases. Tropism for host M/M cells is a common characteristic in these diseases. Sera from naturally infected, and possibly HIV-immunized, individuals have been shown to contain infection enhancing antibodies *in vitro*. Finally, there is considerable genetic, and potentially antigenic, diversity among HIV-1 isolates.

This workshop was convened to evaluate these concerns regarding ADE of HIV infection in human HIV vaccine trials and to propose studies that would address this potential risk. Although there is currently no evidence that immune-mediated enhancement of disease occurs in HIV, there is clearly a need for carefully designed experiments to further evaluate this issue. As there are several notable diseases for which *in vitro* ADE does not correlate with ADE *in vivo*, *in vitro* data are insufficient to deter development of current HIV-1 vaccine candidates. *In vivo* correlates of protection/enhancement are necessary to evaluate the ADE risk accurately. The development of an HIV animal model that would allow testing of vaccine candidates is of primary importance.

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PARTICIPANTS

Donald Burke (Walter Reed Army Institute of Research), Francis Ennis (University of Massachusetts Medical Center), Michael Fanger (Dartmouth Medical School), Patricia Fast (Division of AIDS, NIAID), Scott Halstead (The Rockefeller Foundation), Carl Hanson (California Department of Health), Shibo Jiang (The New York Blood Center), Philip Johnson (Ohio State University), Pauline Jolly (University of Alabama), Nzeera Ketter (Division of AIDS, NIAID), Srisakul Kliks (University of California, San Francisco), Dennis Klinman (Food & Drug Administration), Dale Lawrence (Division of AIDS, NIAID), John Mascola (Walter Reed Army Institute of Research), Bonnie Mathieson (Division of AIDS, NIAID), Thomas Matthews (Duke University Medical Center), James McNamara (Division of AIDS, NIAID), William Mitchell

(Vanderbilt University School of Medicine), Lynne Mofenson (NICHD, NIH), David Montefiori (Vanderbilt University School of Medicine), Ronald Montelaro (University of Pittsburgh), Michael Murphey-Corb (Delta Regional Primate Research Center), Michael Nabors (Johns Hopkins University Hospital), A. Robert Neurath (The New York Blood Center), Barbara Potts (Repligen Corporation), Alfred Prince (The New York Blood Center), W. Edward Robinson (University of California, Irvine), Fred Scott (Cornell Feline Health Center), Alan Schultz (Division of AIDS, NIAID), Herbert Smith (Food & Drug Administration), Gregory Spear (Rush-Presbyterian-St. Luke's Medical Center), Mark Wainberg (McGill University), Mary Clare Walker (Division of AIDS, NIAID), Philip Zack (Walter Reed Army Institute of Research), Susan Zolla-Pazner (NYU Medical School).

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Address reprint requests to:
John R. Mascola
Division of Retrovirology
Walter Reed Army Institute of Research
13 Taft Court
Rockville, Maryland 20850